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*NITROGEN-15 MAGNETIC RESONANCE SPECTROSCOPY, I. CHEMICAL SHIFTS**

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Except for the original determination[1] of nuclear moments, nitrogen magnetic resonance spectroscopy has been limited to the isotope of mass number 14. Although N^{14} is an abundant isotope, it possesses an electric quadrupole moment, which seriously broadens the resonances of nitrogen in all but the most symmetrical of environments.[2] Consequently, nitrogen n.m.r. spectroscopy has seen only limited use in the determination of organic structure. It might be expected that N^{15}, which has a spin of $^1/_2$ and no quadrupole moment, would be very useful, but the low natural abundance (0.36%) and the inherently low signal intensity (1.04×10^{-3} that of H^1 at constant field) have thus far precluded utilization of N^{15} in n.m.r. spectroscopy.[3]

Resonance signals from N^{15} have now been obtained from a series of N^{15}-enriched (30–99%) compounds with a Varian model 4300B spectrometer operated at 6.08 Mc/sec and 14,100 gauss. The samples were contained in 10-mm tubes and were spun for best resolution. The power levels were limited by the rather long relaxation times of N^{15}; however, the signals could be readily observed in the absorption mode. Chemical shifts (Table 1) were measured by the sideband technique with respect to a sample of 8.57 *M* nitric acid which contained a trace of ferric chloride, but are reported with respect to anhydrous ammonia which is a more reproducible standard. Calibration was made either by the substitution method, or by placing the standard in a 5-mm tube concentric to the sample tube. Under conditions of optimum resolution (1 cps), a well-developed ring-out was observed from the resonance of the standard.

Table 1 shows the N^{15} chemical shifts measured so far. As expected, there is general agreement with shifts from N^{14} magnetic resonance studies[1-4] of predominantly inorganic compounds.

For interpretation, chemical shifts are usually divided into diamagnetic and paramagnetic contributions.[5] Diamagnetic shielding is taken to arise from rotation of the electron cloud around the nucleus at the Larmor angular velocity, whereas paramagnetic effects are taken to arise from deviations from idealized symmetry, i.e., increases of orbital angular momentum. Holder and Klein[3c] and Schmidt, Brown, and Williams[4] have interpreted N^{14} chemical shifts in this manner. The

TABLE 1

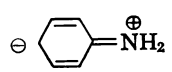
NITROGEN-15 CHEMICAL SHIFTS

Compound	Solvent	Chemical shift, ppm[a] downfield
Ammonia (anhydrous)	None	0
Methylamine	None	2
Ammonium chloride	Water	24
Methylammonium chloride	Water	28
Glycine[b]	Water	31
Anilinium iodide	Water	51
Anilinium tetrafluoroborate	Water	53
Anilinium chloride	Water	55
Aniline	None	59
Methyl isothiocyanate	None	93
Acetanilide	Acetone	135
Potassium cyanide	Water	279
Benzal methylamine	None	325
trans-Azoxybenzene[c,d]	Ether	324 328
Conjugate acid of *trans*-azobenzene[c]	Acid[e]	360
Nitric acid, 8.57 *M*	Water	367
Nitrobenzene	None	372
trans-Azobenzene[c]	Ether	510
Sodium nitrite	Water	608

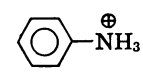
[a] Determined to a precision of about 1%. [b] Unpublished results of B. W. Roberts. [c] Doubly labeled with N^{15}. [d] J (N^{15}-N^{15}) = 14 cps. [e] 65% concentrated sulfuric acid, 20% ethanol, and 15% water.

paramagnetic term is at a minimum for the spherically symmetrical ammonium ion, which therefore produces resonance at high field. Electronic asymmetry may arise either from unshared electron pairs, which cause an upfield shift, or from electronegative substituents, which cause a downfield shift. The paramagnetic shift associated with increases in orbital angular momentum so produced is particularly important in connection with nitrogen chemical shifts.

One of the factors governing the magnitude of the paramagnetic effect is conjugation. Changes in N^{15} chemical shifts resulting from protonation illustrate this point. The data of Table 1 show that protonation can cause both downfield (ammonia and methylamine) and upfield (*trans*-azobenzene) shifts, or it can have almost no effect (aniline). The "normal" shift, 24–26 ppm for ammonia or methylamine, is downfield because protonation removes shielding by the unshared pair. To a degree, this effect should be important for the N^{15} resonance of unprotonated aniline because of delocalization of the unshared pair over the aromatic ring by conjugation (I). Protonation (II) would, of course, remove the shielding by the unshared pair, but this would be a smaller effect than normal because of delocalization. The effects seem fortuitously to cancel, the chemical shift difference on protonation of aniline being only about 6 ppm. The 20 ppm difference between



I



II

methylammonium and anilinium ions is probably due to the inductive and magnetic anisotropy effects of the phenyl ring. That the apparent lack of chemical shift with protonation is not an artifact of the gegenion[6] is indicated by the identity of the shifts in the chloride, tetrafluoroborate, and iodide salts (Table 1).

Since paramagnetic contributions to the chemical shift are inversely proportional to the mean electronic excitation energy,[5] nuclei involved in multiple bonds should

resonate at lower fields, as is the case with carbonyl and olefinic C^{13} resonances.[7] In agreement with this, the N^{15} resonance of *trans*-azobenzene comes at extremely low field. However, protonation causes an upfield shift of 150 ppm. The large change can be explained as an increased mean electronic excitation energy, provided the $n \rightarrow \pi_1^*$ transition makes the dominant contribution. The reason is that only this transition gives a hypsochromic shift when *trans*-azobenzene is protonated.[8] The upfield shift of *trans*-azoxybenzene with respect to *trans*-azobenzene may also be associated with a hypsochromic shift of the $n \rightarrow \pi_1^*$ transition, which, however, has not been unambiguously assigned.[8]

Coupling of N^{15} with directly bonded atoms other than hydrogen has been generally found to be small. A number of these couplings will be discussed in a later paper. A typical example is afforded by $J(N^{15}\text{-}N^{15})$ which was observed to be only 14 ± 1 cps in *trans*-azoxybenzene.

Studies are in progress to utilize N^{15} magnetic resonance spectroscopy as a means of elucidating the molecular and electronic structures of nitrogen compounds, including substances of biological interest.

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*AROMATIC INFLUENCES ON THE YIELDS OF MAMMARY CANCERS FOLLOWING ADMINISTRATION OF 7,12-DIMETHYLBENZ(a)ANTHRACENE**

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Under simple conditions which are easily satisfied, a single dose of 7,12-dimethylbenz(a)anthracene[1] induces mammary cancer in rat selectively, invariably, and rapidly.[2, 3] In this paper the stoichiometry of dose of 7,12-DMBA, and of frequency of its administration as well, are related to yield of mammary cancers under conditions wherein each dose of hydrocarbon evoked cancer in every rat and all doses were well tolerated by the recipient.